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Traditional yogurt dilemma; rich flavor vs. microbial safety: An investigation on Volatile Aroma Profiles, Chemical, and Microbiological Qualities of Traditional Yogurts

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ABSTRACT

This study aimed to bring out chemical, physical, and microbiological quality together with volatile aroma profiles of traditional yogurts collected from the highlands of Ordu and Giresun cities in the Black Sea Region (Turkey). For this purpose, 24 traditional yogurts, 20 of which were produced from cow milk and the remaining were produced from buffalo milk, were collected and analyzes were performed. The average dry matter, protein, fat, pH, total acidity (lactic acid%), viscosity (10 °C), and syneresis values for cow milk and buffalo milk yogurts were 12.68 vs. 14.44%, 3.51 vs. 4.13%, 4.51 vs. 6.55%, 3.80 vs. 3.78, 1.32 vs. 1.60%, 241.09 vs. 1009.21 cP, and 17.43 vs. 9.02%, respectively. The buffalo milk yogurts had higher dry matter, protein, fat, viscosity but lower syneresis values compared to those of cow milk yogurts. The lactic acid bacteria counts were under the required number of 10^7 for cow yogurts while yeast & mould counts were over 10^5 for both cow and buffalo yogurts. Moreover, five of the cow yogurts were found to have coliforms and one being contaminated with *Escherichia coli* indicating unhygienic production conditions. All yogurts contained acetaldehyde, acetoin, ethanol, hexanoic acid, octanoic acid, hexanal, 2-Heptanaone, and 2-Nonanone while diacetyl could not be detected in any yogurt samples. Regarding their compositional values, homemade yogurts were acceptable however they were not appropriate for consumption microbiologically and require improved hygienic conditions for healthier products.

Keywords: Chemical composition, aroma compounds, flavour, fermented milk, homemade yogurt, microbiological quality

INTRODUCTION

Yogurt is an ancient fermented dairy product consumed all over the world today. It is an acid curd produced through fermentation of lactose into lactic acid using two specific bacteria, namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Turkish people are not only known as the inventors of yogurt but also the eponym for the word “yogurt”. As years passed by, yogurt has become a staple part of Turkish food culture and daily nutrition. Nowadays, Turkey is one of the largest producers and consumers of yogurt in the world with an annual yogurt consumption of 30 kg per capita approximately. In Turkey 96% of households consume yogurt, both industrial and home-made yogurts are available while the latter is more common in rural areas (Ünsal, 2019).

Homemade yogurts can be produced for either personal consumption or sale purpose. In homemade yogurt production, previous good quality yogurt is used to inoculate each new batch (backslopping) of boiled and cooled milk in home-scale production followed by incubation until milk gets coagulated, usually for overnight in a warm place inside the house (beside heater or sometimes the containers are covered with blanket or towels to provide adequate temperature for fermentation).

On the other hand, industrial production requires commercial yogurt starter cultures to ferment pasteurized and cooled milk until a pH of 4.6 is obtained under incubation at 42-43 °C. According to a survey, people making their own yogurts in rural areas and cities constitute 77.4% and 46.3% of the total populations, respectively (Republic of Turkey Ministry of Health, 2014). This survey indicates that beside rural consumers, there is a considerable urban population who avoid consuming industrial yogurt therefore making their own yogurts at home. In addition to knowing the sources of milk base and yogurt culture, the sense of confidence that no additives are present can be considered as the primary reasons why consumers avoid industrial yogurts (Gezginç et al., 2018).

Flavor and texture are the characteristics that determine the overall quality and consumer preference in yogurt. They are dependent on the milk base properties (acidity, composition, number of microorganisms, etc.), yogurt culture, and processing conditions such as heat treatment, homogenization, incubation temperature and time (Soukoulis et al., 2007). Although yogurt is mainly produced from cow milk, buffalo milk is also used as a milk base, especially in Mediterranean and Middle East countries, Pakistan, India, and Bangladesh. In Turkey, buffalo milk is commonly used for *kaymak*, a creamy traditional dairy product, however, it is also used in yogurt making (Murtaza et al., 2017). The processing conditions can be standardized under industrial conditions however, their control is almost impossible at home conditions. Thus home-made yogurts do not possess standard qualities. However, traditional home-made yogurts exhibit different advantages from a consumer point of view. Traditional yogurts have pros and cons in terms of sensory properties. Compared to industrial yogurt, traditional yogurt does not have a consistent texture, but its taste and smell are more appreciated by the consumers. Above all, traditional yogurts may carry microbiological risks while industrial conditions provide consumers with standard quality and microbiologically safe yogurt. Mason et al. (2015) determined aflatoxin M_1 concentration to be significantly higher in homemade yogurts than those of industrial ones.

The primary aim of this study was to unveil the chemical, physical, and microbiological quality of traditional yogurts collected from the highlands of the Black Sea region in Turkey. Secondary, the study also gives a perspective on the effects of the milk type, cow or buffalo, on the characteristics of yogurt. Finally, the volatile compounds of traditional yogurts were also investigated.

MATERIALS AND METHODS

Sample collection

Yogurt samples were collected in the summer of 2017 from a variety of high lands and plateaus located in the Black Sea region, Turkey (Figure 1). The sample collection covered two neighboring cities; Ordu (17 samples) and Giresun (7 samples) mainly due to ongoing transhumance culture and lack of studies focusing on these two cities. The locations and corresponding codes are listed in Table 1. The sample collection consisted of 24 yogurts, 20 of which were produced from cow milk and the remaining 4 were produced from buffalo milk. Special care was taken to ensure that all yogurts were produced by traditional means and did not contain any commercial yogurt starter cultures. All yogurts (≈ 250 g) were collected in pre-sanitized glass jars and brought to the lab under cold conditions by using insulated thermal boxes containing ice packs. After securing enough sample for the microbiological analysis and back up (stored at -20 °C), firstly pH and total acidity analyses were performed.

Chemical analysis

A benchtop pH meter (SevenCompact S210, Mettler Toledo, Switzerland), calibrated with pH 4.0 and 7.0 buffers, was used to determine the pH values of yogurts (Kurt et al., 1996). After diluting with distilled water (1:1), yogurt samples were titrated with a standardized solution of 0.1 N NaOH accompanied by phenolphthalein indicator to determine the total titratable acidity as lactic acid (LA%). The diluted yogurts were used to determine the fat contents, and the designated fat percentage on the milk butyrometer was doubled to calculate the total fat concentration (%) using the Gerber-van Gulik method.

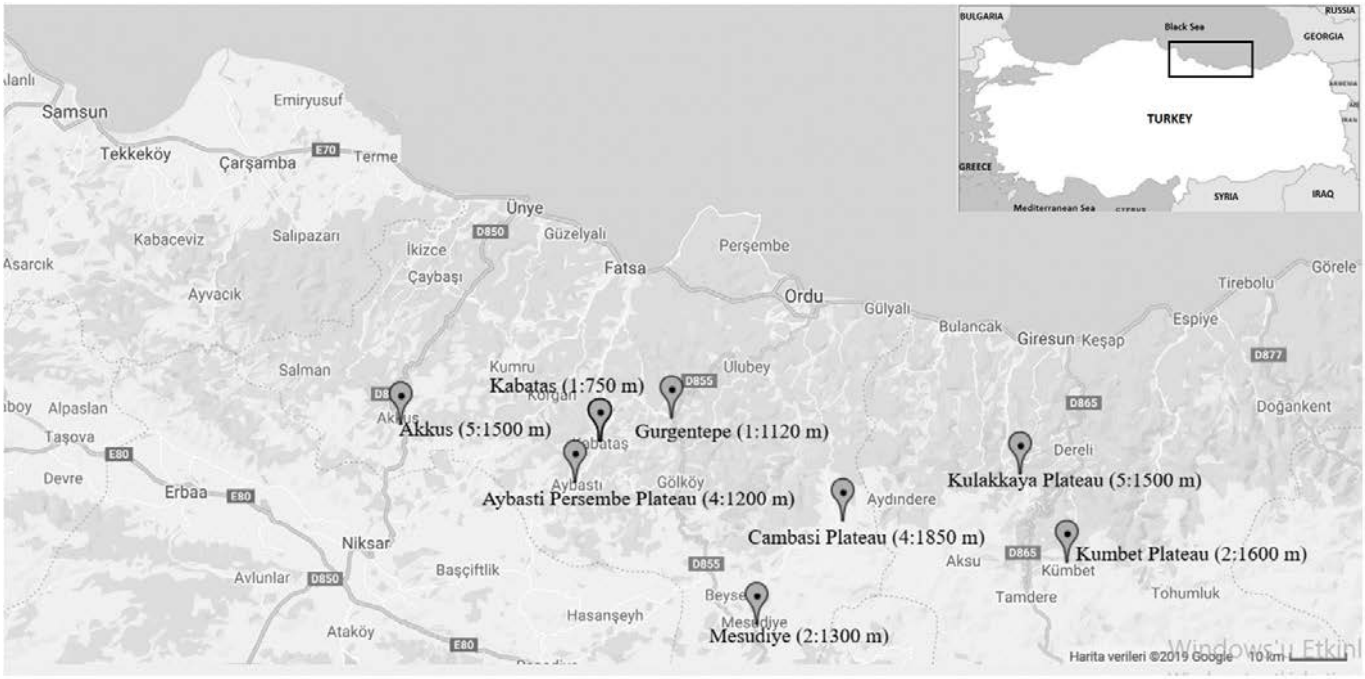

Figure 1. Locations where yogurt samples were collected. Numbers in brackets indicate number of samples and height of location in meters, respectively

Total dry matter was determined gravimetrically based on the method given by IDF (2004). The protein content of the samples was determined based on the Kjeldahl Method using UDK-149 (VELP Scientifica, Italy) automatic distillation unit (IDF/ISO, 2014).

Viscosity and syneresis

The viscosity of yogurt samples was measured at 10 °C and 15 °C, common consumption temperatures for yogurt, using a sine-wave Vibro Viscometer (SV-10, A&D Inc., Japan) which measures the driving electric current required to resonate the two sensor plates at a constant frequency. Syneresis was determined based on the method given by Temiz and Dağyıldız (2017) with modifications. Yogurt samples (10 g) were centrifuged (Model 2-6, Sigma, Germany) at 2,500 rpm for 10 minutes, and syneresis values were calculated as the weight of excreted supernatants per 100 g yogurt.

Microbiological analysis

Samples of 10 g of yogurt were weighed aseptically into sterile bags. After addition of 90 ml of sterile NaCl solution (0.85%, w/v), bags were homogenized for 30 seconds in a stomacher type laboratory blender (BagMixer 400P, Interscience Co., France). Serial dilutions were prepared (1:9) and appropriate dilutions were plated on specific media. Lactic acid bacteria were enumerated according to IDF/ISO (2003). Cycloheximide was also included (0.1 g/L) to suppress the growth of fungi and increase the selectivity of the media. Coliform bacteria count was determined using the Florocult Violet Red Bile Agar (F-VRBA) where lactose positive coliform bacteria form red colonies. The colonies that fluorescence under UV light (365 nm) were identified as *Escherichia coli* among these red colonies. All medium types, plating methods, and incubation conditions are listed in Table 2. All media were purchased from Merck, Germany. The plates containing 30 to 300 colonies were considered for calculation.

Determination of volatile aroma compounds

The analysis was carried out according to the method given by Temiz and Tarakçı (2017) with modifications. A gas chromatography mass spectrometer system (GCMS-QP2010, Shimadzu Corp., Kyoto, Japan) was utilized to determine aroma compounds present in the yogurt samples. The system was equipped with a chromatographic column (Restek, Bellefonte, PA, USA) in

Table 1. The city, plateau, and locations where each yogurt sample is collected and the respective encodings

City	Plateau/High Land	Location/Type	Yogurt Sample Code
ORDU (17)	Akkus	Tanismalaga	AK-T
		Merkez	AK-A
		Iskenderli	AK-I
		Cayiralan/Belalan	AK-C
		Domuzkeli	AK-D
	Aybastı Perşembe	Merkez (A)	P-A
		Merkez (B)	P-B
		Esenli Obasi	P-H
		Esenli Obasi	P-Z
	Cambasi	Merkez	C-C
		Merkez	C-Y
		Susuz Oba (Buffalo)	C-Buf*
		Baskoy	C-B
	Mesudiye	Daricabasi Mahallesi	M-S
		Gelgeser Koyu	M-G
	Gurgentepe	Agizlar Mahallesi	GG
	Kabatas	Kabatas	Kabatas
GIRESUN (7)	Kumbet	Inek	K-I
		Buffalo	K-Buf*
	Kulakkaya	Inek	KK-I
		Inek 2	KK-I2
		Buffalo	KK-Buf*
		Buffalo 2	KK-Buf 2*
		Melikli	KK-Melikli

Asterisks (*) indicate the buffalo milk yogurts

Table 2. Microbiological analysis method details for enumeration of indicated microorganisms

Microorganism	Medium	Plating	Incubation conditions
Coliform bacteria and *E. coli*	Florocult Violet Red Bile Agar (F-VRBA)	Spread	37 °C, 18-24h, aerobic
Total mesophilic aerobic bacteria (TMAB)	Plate Count Agar (PCA)	Spread	30 °C, 48-72h, aerobic
Yeast & mould	Yeast extract glucose chloramphenicol agar (YGC)	Spread	30 °C, 48-72h, aerobic
Lactobacilli	de Man, Rogosa and Sharpe agar (MRS)	Pour	37 °C, 48-72h, anaerobic
Streptococci	M 17 Agar (M17)	Pour	37 °C, 48h, aerobic

60 m length, 0.25 µm thickness, and 0.32 mm diameter. Helium was used as the carrier gas with a flow rate of 3 mL/min and a pressure of 124.2 kPa. The temperature of the column was held at 40 °C for 1 min, raised by 7 °C/min to 100 °C held for 5 min, increased by 4 °C/min to 130 °C held for 1 min, increased by 2 °C/min to 180 °C held for 1 min, and finally, increased by 15 °C/min to 250 °C held for 10 min. The ion source and interface temperatures were 200 °C and 230 °C, respectively. The mass range was *m/z* 35–450 and a scan speed of 1666 were set. Briefly, 2 g of yogurt was transferred to 20 mL screw-cap vials and solid phase microextraction (SPME, 2 cm–50/30 mm DVD/Carboxen/PDMS Stable Flex Supelco, Bellefonte, PA, USA) fiber was inserted into its septum. The vials were held in a water bath set to 60 °C for 63.74 minute which corresponds to the duration of a single sample run in the GCMS system. The identification of volatiles was done by comparison of data with those of in WİLEY 229, NIST, and FFNSCN (Flavor and Fragrance Natural and Synthetic Compounds) libraries. For each sample, the average of two single runs is given. Based on the peak areas, the concentrations of each aroma compound was expressed as the relative abundance (%) of total volatile compounds isolated from the yogurt samples.

Statistical analysis

All analyses were performed in duplicate. Primarily, data were subjected to variance test (Levene test) to decide on the assumption of equal variances for the independent samples t-test. Then, independent samples t-test was applied to determine whether the region and the milk base had a significant effect on the tested quality parameters.

RESULTS AND DISCUSSION

Chemical analysis

Dry matter, fat, and protein contents of cow milk yogurts and buffalo milk yogurts are given in Table 3 and 4, respectively. The dry matter contents ranged between 10.17 and 16.65% with an average value of 12.92% while neither location nor milk type had a significant effect on the dry matter contents (P>0.05). However, buffalo milk yogurts still had higher dry matter content (14.44%) than cow milk yogurts (12.68%). The average protein content of the yogurt samples is 3.63% and this value complies with the minimum value of 3% set for yogurts by the Turkish Food Codex (2009). Milk type affected the protein content significantly (p=0.010) and consequently, buffalo milk yogurts had higher protein content (4.13%) compared to their cow milk counterparts (3.51%).

The fat content of cow milk yogurts had a broad range (2.3-7.0%) with an average content of 4.51% while buffalo milk yogurts had an average fat content of 6.55%. Both cow and buffalo yogurts can be classified as full-fat yogurt, given the fat content higher than 3.8% the yogurts could be classified as full-fat (Turkish Food Codex, 2009). Buffalo milk yogurts had significantly higher (p=0.009) fat content than those of cow milk yogurts. Confirming these findings, Erkaya and Şengül (2011) reported higher total solids (17.87 vs. 12.12%), protein (4.67 vs. 3.61%), and fat (8.40 vs. 4.05%) for buffalo milk yogurts compared to cow milk yogurts. These compositional differences could be mainly attributed to the compositional differences in the milk bases of cow and buffalo species (Rafiq et al., 2016). Location and milk type did not have a significant effect on the pH values (P>0.05). The pH values of the yogurts were mostly below 4.0 with an average of 3.79 which is not in the optimum pH range (4.2-4.6) for fermentation (Table 3 and 4). Cow and buffalo yogurts had very similar pH values of 3.80 and 3.78, respectively. Consistently, the titratable acidity values were high (0.80-1.93%). Although average acidity value for cow milk yogurt is within the limits (0.60-1.50%), average acidity value (1.60%) of buffalo milk yogurts was over the limit assigned by the Turkish Food Codex (2009).

The current average values for all yogurts were found to be higher than the dry matter (13.02%) and fat (3.88%) concentrations but lower than the protein (3.87%), total acidity (3.65%), and pH (3.81) values reported for 25 traditional yogurts collected from Erzurum and Kars cities (Turkey) by Biberoğlu and Ceylan (2013).

Table 3. Gross composition (%), pH and total titratable acidity (%) values for cow milk yogurt samples

Samples	DM (%)	Fat (%)	Protein (%)	pH	TA (LA%)
AK-T	14.47	6.40	3.83	3.61	1.12
AK-A	11.51	3.90	3.00	3.50	1.28
AK-I	10.33	3.10	2.81	4.03	0.80
AK-C	13.87	4.60	3.69	3.83	1.10
AK-D	11.78	3.90	3.48	3.58	1.38
P-A	14.69	5.30	3.85	3.88	1.41
P-B	14.80	5.90	3.26	3.84	1.31
P-H	16.22	7.00	4.31	4.09	1.35
P-Z	16.65	4.30	3.74	4.34	0.94
C-C	11.22	5.50	3.64	3.54	1.58
C-Y	13.70	5.30	3.97	4.22	0.83
C-B	10.26	3.40	3.44	3.53	1.63
M-S	11.67	2.30	3.67	4.07	1.33
M-G	12.80	5.40	2.66	3.62	1.06
GG	11.60	5.30	3.43	3.61	1.61
Kabatas	11.05	2.50	3.57	3.98	1.08
K-I	11.60	4.90	3.11	3.79	1.37
KK-I	11.95	3.70	3.60	3.70	1.69
KK-I2	13.24	5.00	3.70	3.64	1.84
KK-Melikli	10.17	2.50	3.48	3.62	1.62
Max.	16.65	7.00	4.31	4.34	1.84
Min.	10.17	2.30	2.66	3.50	0.80
$\overline{x}$	12.68	4.51	3.51	3.80	1.32

DM: Dry matter, TA (LA%): Titratable acidity (as lactic acid %)

Moreover, Demirkaya and Ceylan (2013) determined the pH (3.84-4.80), acidity (0.72-1.17%), fat (3-4.20%), dry matter (11.25-16.05%), protein (2.65-4.21%), and syneresis (20-46%) values of 30 traditional yogurts collected from Bilecik (Turkey). In another study, 50 traditional yogurts collected from mountain villages of seven cities in Turkey, average total non-fat solids concentrations were under the limits while fat and protein concentrations were found to be proper according to Turkish Standard (TS 1330) regulations (Herdem, 2006). Also, Türkoğlu et al. (2003) reported lower values for dry matter (10.86%), fat (2.93%), and protein (3.38%) concentrations as well as lower values of pH (3.68) and acidity (1.25) for 20 yogurts collected from Sanliurfa, Turkey. Compared to the current values found for buffalo milk yogurts, Bayram (2013) found higher dry matter (17.37%), fat (7.31%), and protein (5.04%) concentrations but lower syneresis values (8.64%).

Table 4. Gross composition (%), pH and total titratable acidity (%) values for buffalo milk yogurt samples

Samples	DM (%)	Fat (%)	Protein (%)	pH	TA (LA%)
C-Buf	13.99	5.40	4.55	3.62	1.93
K-Buf	13.66	6.10	3.71	3.83	1.37
KK-Buf	13.66	7.90	3.82	3.91	1.50
KK-Buf2	16.44	6.80	4.44	3.74	1.60
Max.	16.44	7.90	4.55	3.91	1.93
Min.	13.66	5.40	3.71	3.62	1.37
$\overline{x}$	14.44	6.55	4.13	3.78	1.60

DM: Dry matter, TA (LA%): Titratable acidity (as lactic acid %)

Due to the processing, incubation and storage conditions, yogurt usually undergoes two primary defects physically; variation in viscosity and syneresis (Hematyar et al., 2012). Considering variations in the said parameters in home-scale yogurt production, it would not be rational to expect stable and perfect viscosity and syneresis values from the yogurt samples. Mainly dry matter, more specifically the protein content, has an important role in the viscosity and syneresis values. The higher protein content in yogurt results with higher firmness and higher resistance of yogurt gels against syneresis (Soukoulis et al., 2007). However, together with protein concentration the casein to whey ratio (C:W) plays key role on viscosity and syneresis. For example, Kücükcetin (2008) reported that as C:W increased from 1.5 to 4 the syneresis increased from 67.2% to 79.2% indicating that increasing C:W in yogurts results in higher syneresis. Moreover, yogurts with protein contents higher than 5.6% has been identified as too firm with an astringent flavor by panelists in a study by Mistry and Hassan (1992). Therefore, increasing the protein concentration in yogurt may not always be a solution for better physical and/or sensory characteristics.

Considering that commercial yogurts usually have a 14-15% milk solids (Lucey and Singh, 1997), the relatively low viscosities in homemade cow milk yogurts could be attributed to the low mean dry matter concentration (12.68%) primarily. On the other hand, buffalo yogurts exhibited considerably higher mean viscosity values compared to cow yogurts (1009.21 vs. 241.09 cP) likely as a result of relatively higher protein and dry matter concentrations (Table 5 and 6). The viscosity value of buffalo yogurts are close to the values reported by Han et al. (2012) for plain buffalo yogurt (1400-1780 cP). However, current values are quite low compared to the values of 8944 and 2339 cP reported by Hanif et al. (2012) for cow and buffalo yogurts, respectively. The viscosity differences between the studies is probably a result of variation in dry matter contents of yogurts although it was not reported. Involvement of homogenization process in the latter study could be another explanation for the high viscosity since homogenization enhances the interactions between protein and fat globules thereby increasing the viscosity (Nguyen et al., 2014; Özer, 2006; Sfakianakis and Tzia, 2014). The low total solids content, excessive heat treatment of the mix, and rapid acidification rate may be considered as the main reasons for the occurrence of syneresis in homemade yogurts among the possible causes of whey separation as listed by Lucey and Singh (1997).

Microbiological analysis

Yogurt gel is formed through the coagulation of milk proteins due to the lactic acid produced through fermentation of lactose in milk by acting bacteria in yogurt culture. Given that, each gram of yogurt must

contain at least 10^7 cfu of both yogurt bacteria, namely *L. bulgaricus* and *S. thermophilus*, at the time of consumption (Turkish Food Codex, 2009). The average lactobacilli and streptococci counts were $<10^7$ in the cow milk yogurts whereas they are $>10^7$ for buffalo milk yogurts. Among all, five cow milk yogurt samples (AK-T, AK-I, P-H, P-Z, and C-Y) were found to contain coliform bacteria while *E. coli* was detected in one of them (P-H). The yeast & mould counts of both cow and buffalo yogurts were also quite high (2.85-7.48 log cfu/g) with averages of 5.54 and 6.03 log cfu in per gram of yogurt, respectively. The average total aerobic mesophilic bacteria count was determined as 7.81 and 8.19 log cfu for per gram of cow and buffalo yogurts, respectively (Table 7 and Table 8).

Homemade yogurt production involves the boiling of raw milk for an uncertain time, usually a long time (up to 2 hours), to maintain the sterility and consistency of the yogurt. In home scale, incubation time (depending on the season from 2 to 12 hours) and temperature also are uncontrolled usually ending up with low pH values. Thereby, due to severe heat application and high acidity, the presence of coliforms, yeasts, and moulds is most likely a result of unhygienic conditions and contamination afterward. Hisoğlu (2007) found that 57.94% and 14.95% of 107 homemade yogurts collected in Agri province (Turkey) do not comply with the yeast & mould and *E. coli* numbers set by the standards. In another study on homemade yogurts, yeast & mould counts were way high while some of the yogurts even did not contain any yogurt bacteria (Herdem, 2006). Durak et al. (2008), collected 20 homemade yogurts in Konya province (Turkey) and determined that coliform and yeast & mould numbers were above the standards in 80% of the yogurts with the average counts of 5.48 log cfu/g and 4.63 log cfu/g, respectively. Similarly, Demirkaya and Ceylan (2013) also found that coliform and yeast & mould counts of 3.33% and 66.67% of 30 yogurts procured in Bilecik province were not appropriate for consumption, respectively.

Table 5. Viscosity at 10 °C and 15 °C (cP) and syneresis (%) values for cow milk yogurt samples

Samples	Viscosity (10 °C)	Viscosity (15 °C)	Syneresis (%)
AK-T	436.36	311.09	10.09
AK-A	234.83	164.5	27.56
AK-I	116.86	77.36	19.73
AK-C	355.48	239.46	5.52
AK-D	151.58	108.12	14.33
P-A	130.35	92.54	22.23
P-B	95.01	72.78	10.76
P-H	414.42	296.87	10.77
P-Z	221.92	165.89	17.57
C-C	213.93	161.55	7.46
C-Y	385.37	301.12	9.04
C-B	129.84	101.22	38.03
M-S	198.51	210.01	26.36
M-G	106.7	79.02	43.34
GG	192.54	141.23	8.30
Kabatas	63.37	45.02	25.71
K-I	54.78	46.52	16.73
KK-I	444.01	339.58	5.41
KK-I2	519.33	461.18	5.37
KK-Melikli	356.69	260.31	24.20
Max.	519.33	461.18	43.34
Min.	54.78	45.02	5.37
$\overline{x}$	241.09	183.77	17.43

Table 6. Viscosity at 10 °C and 15 °C (cP), and syneresis (%) values for buffalo milk yogurt samples

Samples	Viscosity (10 °C)	Viscosity (15 °C)	Syneresis (%)
C-Buf	1771.96	1407.06	6.72
K-Buf	355.45	326	17.46
KK-Buf	453.97	399.81	0.82
KK-Buf2	1455.45	1077.36	11.08
Max.	1771.96	1407.06	11.08
Min.	355.45	326	6.72
$\overline{x}$	1009.21	802.56	9.02

Table 7. Microbiological properties (log cfu/g) of cow milk yogurt samples

Samples	Coliform	*E. coli*	TMAB	Yeast & Mould	Lactobacilli	Streptococci
AK-T	6.20	ND	7.61	6.90	6.18	7.60
AK-A	ND	ND	6.57	6.68	5.70	6.87
AK-I	2.60	ND	7.90	4.30	5.04	8.20
AK-C	ND	ND	5.48	5.45	5.00	4.74
AK-D	ND	ND	7.48	5.49	4.70	8.15
P-A	ND	ND	8.89	3.36	8.48	6.86
P-B	ND	ND	8.80	2.85	8.61	6.98
P-H	4.83	3.08	8.48	6.58	5.94	5.40
P-Z	5.57	ND	6.79	6.25	6.00	6.77
C-C	ND	ND	7.48	3.48	5.74	6.93
C-Y	3.98	ND	7.96	5.51	6.77	7.82
C-B	ND	ND	7.67	3.30	6.00	5.37
M-S	ND	ND	7.14	7.06	7.15	6.41
M-G	ND	ND	8.03	6.40	6.08	8.04
GG	ND	ND	8.56	5.26	7.18	4.40
Kabatas	ND	ND	8.16	6.29	6.71	5.64
K-I	ND	ND	8.52	5.60	6.08	4.99
KK-I	ND	ND	8.32	7.18	7.49	8.36
KK-I2	ND	ND	8.20	6.32	7.08	5.56
KK-Melikli	ND	ND	8.21	6.56	6.45	7.89
Max.	6.20	3.08	8.89	7.18	8.61	8.36
Min.	0.00	0.00	5.48	2.85	4.70	4.40
$\overline{x}$	1.22	0.15	7.81	5.54	6.42	6.65

TMAB: Total mesophilic aerobic bacteria, ND: Not determined

Moreover, Bayram (2013) detected yeast & mould counts of >5 log cfu for buffalo milk yogurts. In another study on 100 buffalo milk yogurts sold in Kayseri, the average yeast, mould, total mesophilic aerobic bacteria, and lactic acid bacteria counts were, 5.21 log cfu, 5.16 log cfu, 7.72 log cfu, and 6.58 log cfu, respectively (Ertaş et al., 2014).

Determination of volatile aroma compounds

Yogurt aroma was investigated more than the other fermented products with around 100 aroma compounds identified (Cheng, 2010; Routray and Mishra, 2011). The balances between these aroma substances produced by the breakdown of proteins, carbohydrates and fats depending on the type of milk and various processes applied to milk during the production of yogurt determine the acceptability and appreciation of the yogurt. *L.*

Table 8. Microbiological properties (log cfu/g) of buffalo milk yogurt samples

Samples	Coliform	*E. coli*	TMAB	Yeast & Mould	Lactobacilli	Streptococci
C-Buf	ND	ND	7.39	3.70	6.51	7.13
K-Buf	ND	ND	8.85	5.65	7.18	4.83
KK-Buf	ND	ND	8.26	7.48	8.03	8.20
KK-Buf2	ND	ND	8.26	7.48	8.03	8.20
Max.	0.00	0.00	8.85	7.48	8.03	4.83
Min.	0.00	0.00	7.39	3.70	6.51	8.20
$\overline{x}$	0.00	0.00	8.19	6.08	7.44	7.09

TMAB: Total mesophilic aerobic bacteria, ND: Not determined

bulgaricus and *S. thermophilus*, which are used as starter cultures in yogurt production, also contribute to the aroma formation through fermentation. As a result of fermentation of milk by yogurt cultures, various carbonyl compounds such as acetaldehyde, acetone, acetoin, and diacetyl which are thought to be the main aroma components of yogurt together with lactic acid, gives a sour taste and refreshing sensation, and form the typical yogurt flavor (Chaves et al., 2002). In addition to these, depending on the storage time and conditions of the yogurt, various undesirable aroma substances may be formed as a result of microbial activities and biochemical reactions in the yogurt.

In the scope of this study, there were more than 80 volatile compounds identified for each yogurt sample and 17 compounds, corresponding to more than 70% of the total volatiles, were selected from the identified compounds and their proportional concentrations are given (Supplementary Material, Table S1). Hexanoic acid, octanoic acid, ethanol, acetaldehyde, hexanal, acetoin, 2-Heptanaone, and 2-Nonanone were detected in all yogurt samples. On the other hand, benzaldehyde, with an almond like aroma, and 3-methyl-2-butanone were only detected in two of the cow milk yogurts. Moreover, diacetyl (2,3-butanedione) which is thought to be an important compound for yogurt aroma could not be detected in the samples. Diacetyl has an important role in yogurt aroma and the recommended diacetyl: acetaldehyde ratio for a full yogurt aroma is 1: 4 (Beshkova et al., 1998) moreover, it has been reported to complement the aroma of acetaldehyde, especially when acetaldehyde is low in concentration. Among acids, only 2 samples did not contain acetic acid while butyric acid was not detected in 5 yogurt samples. Hexanoic and caprylic acids were detected in all yogurt samples.

The concentration of acetaldehyde, the major volatile compound in yogurt, and the ratio of this component to others are accepted as criteria in the sensory acceptance of yogurt. In this study, when all yogurts are taken into consideration, the amount of acetaldehyde varied between 0.20 and 4.03%, while its concentration was relatively higher in buffalo milk yogurts. Similarly, Erkaya and Şengül (2011) reported that buffalo milk yogurts possessed the highest acetaldehyde concentration compared to its counterparts produced from cow, sheep, and goat milks. Hexanal, another aldehyde, known for its fruity and grassy aroma, has been detected in all yogurts. Acetone and acetoin are ketones that play important roles in yogurt aroma. Acetone (0.30-4.10%) could not be detected in one of the yogurt samples while the acetoin (0.27 to 30.46%) was detected in all samples. Acetaldehyde can be degraded into ethanol by the alcohol dehydrogenase enzyme secreted by yogurt bacteria. Although ethanol formation is possible during yogurt fermentation, the amount of ethanol released is quite low under normal circumstances.

The amount of ethanol in yogurt samples has a wide range from 0.45 to 30.46% (Supplementary material, Table S1). Ethanol formation is usually associated with yeast metabolism (Lourens-Hattingh and Viljoen, 2001) thereby the high ethanol concentration is likely to be due to the high number of mould & yeasts determined in microbiological analyzes of yogurts.

CONCLUSIONS

The traditional yogurts collected from the highlands of the Black Sea region met the criteria set by the Turkish Food Codex in terms of chemical composition. The relatively high acidity values indicate uncontrolled fermentation conditions (time and temperature). Since milk-fat is economically valuable, the high-fat content determined in yogurts could be a response to the demand and expectations of local consumers. Although it is difficult to make a precise statement, considering the unknown processing conditions for yogurts, it could still be speculated that these variations in chemical and physical properties are mainly due to the compositional differences in milk bases of buffalo and cow.

On the other hand, the yogurts were not appropriate for consumption regarding microbiological parameters, namely lactic acid bacteria and yeast & mould counts. These improper microbiological qualities are indicators of insufficient hygienic conditions for the processing equipment, environment, and/or individuals involved in the production. In fact, traditional yogurts are preferred, mainly due to being natural and their unique and strong flavor, consumed by a consumer mass that cannot be underestimated. Therefore, it is required to not only improve the microbiological quality of traditional yogurts but also raise awareness among consumers on the importance of hygiene and inform local producers about possible hazards. Also, further studies are required to identify this microflora involved in traditional yogurt fermentation and bring in these cultures to the industry as potential starter cultures to provide aromatic cultures and offer alternatives to the producers as well as consumers.

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Table S1. Main volatile aroma compounds detected in the traditional yogurt samples (%)

Volatile Compounds	AK-I	AK-A	AK-C	AK-D	AK-T	C-C	C-Y	C-B
Acids								
Acetic acid	17.05 ± 0.74	2.02 ± 0.29	1.92 ± 0.23	2.42 ± 0.22	2.38 ± 0.20	4.54 ± 0.22	12.17 ± 2.61	2.49 ± 0.31
Butanoic acid (CAS) n-Butyric acid	1.00 ± 0.07	0.67 ± 0.22	1.36 ± 0.32	1.11 ± 0.07	0.37 ± 0.05	0.61 ± 0.11	1.70 ± 0.09	0.79 ± 0.12
Hexanoic acid (CAS) n-Hexanoic acid	5.66 ± 0.37	4.88 ± 0.73	5.87 ± 0.81	4.36 ± 0.08	3.10 ± 0.58	5.08 ± 0.26	7.56 ± 0.13	5.20 ± 0.94
Octanoic acid (CAS) Caprylic acid	4.17 ± 0.14	6.18 ± 0.41	5.03 ± 0.44	5.43 ± 0.16	5.53 ± 0.85	7.47 ± 0.32	5.18 ± 0.96	7.41 ± 1.10
Alcohols								
1-Butanol. 3-methyl- (impure) (CAS) 3-Methyl-1-butanol	ND	10.22 ± 0.83	1.76 ± 0.67	2.43 ± 0.19	12.48 ± 1.84	ND	3.06 ± 0.86	0.55 ± 0.00
Ethanol (CAS) Ethyl alcohol	0.84 ± 0.02	19.36 ± 6.09	1.21 ± 0.25	4.91 ± 0.25	30.46 ± 0.79	4.80 ± 0.16	1.32 ± 0.07	2.04 ± 0.25
Aldehydes								
Acetaldehyde (CAS) Ethanal	0.20 ± 0.10	0.50 ± 0.01	1.32 ± 0.26	0.85 ± 0.09	0.50 ± 0.02	2.67 ± 0.14	1.13 ± 0.26	2.00 ± 0.03
Benzaldehyde (CAS) Phenylmethanal	ND	ND	ND	19.51 ± 0.49	ND	ND	ND	ND
Hexanal (CAS) n-Hexanal	9.83 ± 0.64	0.23 ± 0.08	0.38 ± 0.13	0.71 ± 0.02	0.99 ± 0.13	1.61 ± 0.06	3.57 ± 0.11	2.47 ± 0.24
Esters								
Acetic acid ethyl ester (CAS) Ethyl acetate	ND	18.43 ± 1.37	3.23 ± 1.03	0.52 ± 0.07	7.97 ± 1.14	2.94 ± 0.11	3.67 ± 0.23	3.71 ± 0.11
Oct-1-en-3-yl acetate	ND	0.79 ± 0.15	0.38 ± 0.06	1.49 ± 0.04	ND	9.42 ± 0.56	ND	8.05 ± 0.02
Ketones								
2-Butanone (CAS) Methyl ethyl ketone	0.14 ± 0.01	ND	0.31 ± 0.05	ND	ND	ND	0.42 ± 0.00	ND
2-Butanone. 3-hydroxy- (CAS) Acetoin	30.46 ± 0.65	1.65 ± 0.34	4.66 ± 1.66	1.88 ± 0.02	0.76 ± 0.01	1.47 ± 0.04	14.88 ± 2.19	0.49 ± 0.10
2-Butanone. 3-methyl- (CAS) 3-Methyl-2-butanone	7.20 ± 0.59	ND	ND	ND	ND	ND	5.44 ± 0.41	ND
2-Heptanone (CAS) Heptan-2-one	3.49 ± 0.23	5.62 ± 0.64	24.14 ± 5.90	12.33 ± 0.40	6.33 ± 1.00	13.17 ± 0.15	5.63 ± 1.80	14.70 ± 0.46
2-Nonanone (CAS) Methyl heptyl ketone	2.30 ± 0.19	4.13 ± 0.20	12.47 ± 1.87	9.65 ± 0.28	4.36 ± 0.55	16.80 ± 1.00	3.18 ± 0.10	20.13 ± 1.57
2-Propanone (CAS) Acetone	0.92 ± 0.11	0.34 ± 0.02	1.09 ± 0.40	1.14 ± 0.07	0.13 ± 0.01	0.94 ± 0.03	1.90 ± 0.11	0.79 ± 0.03

Values are means ± standard deviations of two replicates. ND: not detected

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Table S1. Continued

Volatile Compounds	C-Buf	GG	Kabatas	K-Buf	K-I	KK-Buf	KK-Buf2	KK-I
Acids								
Acetic acid	3.87 ± 0.07	3.32 ± 0.64	4.16 ± 0.23	ND	0.57 ± 0.05	6.14 ± 0.67	5.83 ± 2.28	2.76 ± 0.13
Butanoic acid (CAS) n-Butyric acid	4.56 ± 0.99	0.91 ± 0.06	ND	1.85 ± 0.29	1.65 ± 0.21	ND	ND	3.73 ± 0.69
Hexanoic acid (CAS) n-Hexanoic acid	17.47 ± 0.65	6.79 ± 0.56	5.10 ± 0.50	6.02 ± 1.64	5.97 ± 0.63	2.83 ± 0.23	0.91 ± 1.28	11.65 ± 1.62
Octanoic acid (CAS) Caprylic acid	11.92 ± 2.28	6.47 ± 0.47	4.18 ± 0.09	3.40 ± 1.18	4.16 ± 0.68	2.41 ± 1.12	0.44 ± 0.63	6.79 ± 0.55
Alcohols								
1-Butanol. 3-methyl- (impure) (CAS) 3-Methyl-1-butanol	2.47 ± 0.02	ND	2.98 ± 0.52	5.08 ± 0.65	10.74 ± 0.65	0.93 ± 0.11	9.02 ± 1.66	2.48 ± 0.54
Ethanol (CAS) Ethyl alcohol	2.98 ± 0.36	0.45 ± 0.01	5.36 ± 0.05	10.11 ± 1.12	25.63 ± 1.27	0.82 ± 0.25	5.05 ± 0.05	3.18 ± 0.56
Aldehydes								
Acetaldehyde (CAS) Ethanal	1.32 ± 0.05	3.12 ± 0.13	3.02 ± 0.11	2.05 ± 0.00	0.20 ± 0.02	4.03 ± 0.16	1.69 ± 0.15	1.72 ± 0.14
Benzaldehyde (CAS) Phenylmethanal	ND	ND	ND	ND	1.69 ± 0.13	ND	ND	ND
Hexanal (CAS) n-Hexanal	0.89 ± 0.47	1.73 ± 0.01	6.10 ± 0.77	0.88 ± 0.09	0.66 ± 0.11	1.11 ± 0.03	0.23 ± 0.10	1.68 ± 0.29
Esters								
Acetic acid ethyl ester (CAS) Ethyl acetate	4.22 ± 0.17	0.93 ± 0.03	1.12 ± 0.09	18.98 ± 1.27	22.03 ± 2.40	6.31 ± 0.83	34.66 ± 5.52	3.88 ± 0.92
Oct-1-en-3-yl acetate	7.1 ± 2.01	3.07 ± 0.45	ND	2.99 ± 0.54	0.70 ± 0.13	ND	ND	2.80 ± 0.12
Ketones								
2-Butanone (CAS) Methyl ethyl ketone	0.2 ± 0.14	ND	ND	ND	ND	0.39 ± 0.14	ND	0.23 ± 0.02
2-Butanone. 3-hydroxy- (CAS) Acetoin	2.11 ± 0.14	3.90 ± 0.01	6.77 ± 1.26	3.62 ± 0.62	0.54 ± 0.05	6.79 ± 0.30	6.85 ± 1.96	2.95 ± 0.64
2-Butanone. 3-methyl- (CAS) 3-Methyl-2-butanone	ND	ND	ND	ND	ND	ND	ND	ND
2-Heptanone (CAS) Heptan-2-one	10.02 ± 2.25	19.95 ± 0.44	10.98 ± 1.46	11.07 ± 0.10	1.75 ± 0.40	12.99 ± 0.09	8.79 ± 0.75	7.07 ± 0.69
2-Nonanone (CAS) Methyl heptyl ketone	10.48 ± 1.19	15.23 ± 1.47	9.59 ± 0.44	11.19 ± 0.21	1.54 ± 0.22	10.94 ± 0.58	7.17 ± 1.00	5.62 ± 0.18
2-Propanone (CAS) Acetone	1.28 ± 0.26	1.52 ± 0.02	4.10 ± 0.25	2.02 ± 0.07	0.14 ± 0.02	2.44 ± 0.14	1.58 ± 0.36	1.35 ± 0.26

Values are means ± standard deviations of two replicates. ND: not detected

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Table S1. Continued

Volatile Compounds	KK-I2	KK-Melikli	M-G	M-S	P-A	P-B	P-H	P-Z
Acids								
Acetic acid	2.30 ± 0.28	3.32 ± 0.76	1.62 ± 0.04	1.00 ± 0.14	4.30 ± 0.52	0.86 ± 0.10	2.17 ± 0.17	ND
Butanoic acid (CAS) n-Butyric acid	0.81 ± 0.08	2.15 ± 0.45	ND	ND	0.70 ± 0.07	1.06 ± 0.15	0.71 ± 0.04	0.23 ± 0.32
Hexanoic acid (CAS) n-Hexanoic acid	2.21 ± 3.13	15.24 ± 1.13	1.00 ± 0.20	1.86 ± 0.01	4.77 ± 0.05	5.08 ± 0.04	4.82 ± 0.13	0.90 ± 1.27
Octanoic acid (CAS) Caprylic acid	1.83 ± 2.58	20.06 ± 3.92	1.66 ± 0.04	4.79 ± 0.22	5.16 ± 0.44	4.52 ± 1.13	5.08 ± 0.96	1.14 ± 1.61
Alcohols								
1-Butanol. 3-methyl- (impure) (CAS) 3-Methyl-1-butanol	5.44 ± 0.71	1.08 ± 0.61	11.17 ± 0.86	4.87 ± 0.60	ND	ND	6.03 ± 0.88	7.72 ± 0.44
Ethanol (CAS) Ethyl alcohol	5.00 ± 0.19	0.78 ± 0.16	4.32 ± 0.42	23.99 ± 4.92	0.86 ± 0.20	0.45 ± 0.03	10.31 ± 1.00	13.37 ± 0.61
Aldehydes								
Acetaldehyde (CAS) Ethanal	0.61 ± 0.12	1.55 ± 0.22	0.41 ± 0.00	0.10 ± 0.02	3.73 ± 0.16	3.52 ± 0.30	2.47 ± 0.11	1.14 ± 0.58
Benzaldehyde (CAS) Phenylmethanal	ND	ND	ND	ND	ND	ND	ND	ND
Hexanal (CAS) n-Hexanal	0.76 ± 0.15	0.39 ± 0.17	0.20 ± 0.03	0.02 ± 0.03	3.71 ± 0.38	4.57 ± 0.28	8.66 ± 0.04	2.37 ± 0.06
Esters								
Acetic acid ethyl ester (CAS) Ethyl acetate	39.60 ± 4.64	2.32 ± 0.72	40.96 ± 0.73	0.34 ± 0.03	ND	ND	22.00 ± 1.89	31.05 ± 1.86
Oct-1-en-3-yl acetate	2.67 ± 0.10	2.56 ± 0.48	2.47 ± 0.03	0.31 ± 0.02	5.16 ± 0.33	ND	ND	ND
Ketones								
2-Butanone (CAS) Methyl ethyl ketone	ND	0.83 ± 0.31	ND	ND	0.38 ± 0.04	0.42 ± 0.03	ND	ND
2-Butanone. 3-hydroxy- (CAS) Acetoin	1.77 ± 0.51	1.45 ± 0.41	1.37 ± 0.14	0.27 ± 0.08	3.60 ± 0.03	3.45 ± 0.04	6.02 ± 0.46	3.26 ± 0.04
2-Butanone. 3-methyl- (CAS) 3-Methyl-2-butanone	ND	ND	ND	ND	ND	ND	ND	ND
2-Heptanone (CAS) Heptan-2-one	7.14 ± 0.65	14.53 ± 2.69	9.39 ± 0.38	1.00 ± 0.02	19.54 ± 2.62	19.86 ± 0.54	4.26 ± 0.10	5.51 ± 0.74
2-Nonanone (CAS) Methyl heptyl ketone	6.86 ± 0.09	10.37 ± 1.34	9.51 ± 0.39	1.41 ± 0.00	17.79 ± 2.28	16.54 ± 1.75	2.84 ± 0.34	3.55 ± 0.38
2-Propanone (CAS) Acetone	0.51 ± 0.01	1.02 ± 0.19	0.51 ± 0.01	ND	2.18 ± 0.19	1.88 ± 0.01	0.32 ± 0.01	1.15 ± 0.07

Values are means ± standard deviations of two replicates. ND: not detected